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Extraintestinal *Campylobacter jejuni* and *Campylobacter coli* Infections: Host Factors and Strain Characteristics

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To determine whether extraintestinal isolates of *Campylobacter jejuni* and *Campylobacter coli* are the consequence of unusual host or bacterial characteristics, we studied clinical and bacteriologic features of 24 extraintestinal infections. Common serotypes and auxotypes were present among the extraintestinal isolates. Gastrointestinal isolates were more susceptible to normal human serum than were the systemic isolates; however, the ranges overlapped considerably. Predispositions to systemic spread were present in 52% of patients with extraintestinal infections; isolates from these patients were more often (73%) serum sensitive than were isolates from patients without predispositions (9%; $P = .002$). By sodium dodecyl sulfate-polyacrylamide gel electrophoresis, no specific protein band was associated with serum resistance, and all isolates of *C. jejuni* and *C. coli* had rough-type lipopolysaccharide profiles. Serum susceptibility was inversely correlated with carbohydrate or ketodeoxyoctonate (KDO) fraction of cell weight and directly correlated with KDO:carbohydrate ratio. Our results suggest that either host defects or specific bacterial virulence characteristics, such as serum resistance, possibly related to length of lipopolysaccharide side chain, may be responsible for extraintestinal infections due to *C. jejuni* and *C. coli*.

Campylobacter jejuni and the closely related *Campylobacter coli* predominantly cause intestinal illnesses [1, 2]. Occasional extraintestinal infections have been reported, but other than case reports [3–6], the characteristics of the infected hosts or the specific organisms have not been examined to a great extent. From the infrequency of reports of systemic infection in comparison with the thousands of intestinal infections reported annually in the United States [7], United Kingdom [8], and other developed countries, it appears that extraintestinal infections are relatively uncommon. Surveillance of *Campylobacter* infections in the United States [7] showed that extraintestinal sources accounted for 26 (0.4%) of 6,402 isolates of *C. jejuni*. An important unanswered question is whether extraintestinal infections due to *C. jejuni* or *C. coli* are the result of a subpopulation of these enteric pathogens that are capable of invading the bloodstream or of compromised host defense capabilities. This report provides information pertinent to both possibilities.

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Materials and Methods

Bacterial strains. Extraintestinal isolates of *C. jejuni* and *C. coli* were either from patients in Denver, were generously sent by investigators who had reported bloodstream or systemic infections in

the medical literature [3–6, 9–11], or were from the culture collection of the Special Pathogens Section of the Centers for Disease Control (Atlanta). Fecal isolates for comparative studies were randomly selected from the *Campylobacter* laboratory culture collection in Denver. From two patients, both blood and fecal isolates were examined. After receipt, strains were passaged three or fewer times on trypticase-soy agar with 5% sheep blood (blood agar; PASCO, Wheat Ridge, Colo) before being studied. All strains were incubated at 42 C for 48 hr in a microaerobic atmosphere, as previously described [12].

Clinical information. Published reports or medical records were reviewed for each patient from whom *Campylobacter* isolates were studied to determine demographic features, predispositions to extraintestinal infections, and nature of illness produced.

Serotyping and auxotyping. Isolates were serotyped by two procedures. The Penner method [13] for heat-stable antigens used 57 unadsorbed hyperimmune antisera in a microtiter IHA procedure. The Penner serotype (PEN) was indicated by listing all reactive antisera in order of strength of titer activity. The antigen giving the highest titer was listed first, and multiple antigens reacting to the same titer were listed in numerical order. Weak reproducible antigens were included in the serotype if one or more strong antigens were present. Nontypable isolates did not react in any of the antisera or showed weak reactions only. The Lior method [14] for heat-labile antigens used liver cells suspended in 0.1% DNase solution in buffer and 55 unadsorbed antisera and antisera adsorbed to remove homologous heat-stable and heterologous heat-labile antibody. The Lior serotype was indicated by the unadsorbed and adsorbed antisera (usually only one) showing agglutination. Nontypable isolates did not agglutinate in any antisera. Rough isolates agglutinated in all or most unadsorbed antisera. Auxotyping was performed as previously described [14a].

Analytical methods. To detect lipopolysaccharide (LPS) structure in whole cells in PAGE, we used the method of Hitchcock with minor modifications as previously described [15, 16]. We resolved total cell proteins by SDS-PAGE, as described [17]. To determine the chemical composition of 31 bacterial strains, we grew 48-hr cultures to confluence on blood agar, harvested cells in water, and after centrifugation we lyophilized and weighed the pellet. Protein concentrations were measured by using the Markwell method, 2-keto-3-deoxyoctonate (KDO) concentrations were measured by using the thiobarbituric acid method, and carbohydrate concentrations were determined by using the phenol-sulfuric acid procedure, all according to previous descriptions [15, 16].

Serum susceptibility. The susceptibility of *C. jejuni* and *C. coli* strains to the bactericidal activity present in normal human serum was assessed in a standardized assay, as previously described [12]. In brief, 24-hr cell cultures were diluted in medium 199 with HBSS to concentrations of 10^3–10^5 cfu/ml, then incubated for 60 min at 37 C with 10% pooled serum from healthy adults. Pre- and postincubation counts were compared in order to calculate $\log_{10}$ killing. On the basis of our previous study [12], serum sensitivity was defined as >1.0 $\log_{10}$ (90%) killing, resistance as <0.1 $\log_{10}$ killing, and intermediate, between these two values; all strains were tested in duplicate.

Statistics. Distributions of values within groups of strains were tested for statistical significance by using one-way (unpaired) analysis of variance or Student's *t* test. Associations between serum susceptibility and chemical characteristics of the strains were examined by using linear-regression analysis.

Results

Clinical characteristics. A total of 24 extraintestinal isolates were studied, 13 from the bloodstream and 11 from other sites. Using the ability to hydrolyze hippurate as the distinguishing characteristic [18], we identified 18 isolates as *C. jejuni* and six as *C. coli* (three bloodstream and three other systemic isolates). We had clinical information relating to 23 of the 24 bloodstream and other systemic isolates. Patients' ages ranged from 12 days to 77 years (median, 26 years); however, children under one year old (eight isolates) and persons ≥60 years (seven isolates) represented 65% of those whose age was known. A potential predisposition to extraintestinal infection was present in 12 (52%) patients, including biliary tract disease (four patients), hypogammaglobulinemia (two patients), first month of life, immunosuppression, previous radiation therapy, chronic renal failure, pregnancy, and aortic prosthesis (one patient each). Six isolates from patients with predisposition to extraintestinal infection were from cultures of blood and six were from other systemic sites (table 1). Of 11 isolates from systemic sites, all were con-

Table 1. Clinical and bacteriologic characteristics of infections due to *C. jejuni* and *C. coli* extraintestinal isolates.

Isolate	Species	Patient age/sex*	Illness	Isolation site	Log_{10} kill†	Serotype‡ PEN	Serotype‡ Lior	Auxotype‖
84-157	*C. coli*	19/F	Septic abortion	Blood	2.24	NT	rough	0
84-23	*C. jejuni*	77/F	Gastroenteritis, transient bacteremia	Blood	0.84	1, 44, 3	2	0
84-26	*C. jejuni*	66/M	Gastroenteritis, continuous bacteremia with abdominal aortic prosthesis	Blood	0.29	16	4	0
84-27	*C. jejuni*	14d/F	Bacteremia, sepsis	Blood	2.56	3, 13w	59	Arg^-
79-263	*C. jejuni*	48/M	Recurrent colitis	Blood§	3.80	4	9	Pro^-
79-193	*C. jejuni*			Feces§	4.02	NT	11	Pro^-
78-64	*C. coli*	25/F	Immunosuppressed, sepsis	Blood	2.52	20	14	0
84-49	*C. jejuni*	26/F	Hypogammaglobulinemia, recurrent diarrhea, bacteremia	Blood	1.16	1	2	Met^-
84-28	*C. jejuni*	26/M	Colitis, persistent asymptomatic bacteremia	Blood	0.19	22, 16w	NT	0
84-101	*C. jejuni*	3m/M	Biliary atresia, obstructive jaundice, sepsis, no diarrhea	Blood	1.17	3, 1w, 8w, 13w	36	0
84-102	*C. jejuni*	6m/M	Bronchiolitis, aseptic meningitis, transient bacteremia, no diarrhea	Blood	0.25	2	4	0
84-133	*C. jejuni*	1/M	Gastroenteritis, transient bacteremia	Blood§	0.22	4, 13, 43w, 3w	1, 24	0
84-66	*C. jejuni*			Feces§	0.55	4, 13	24	ND
84-134	*C. jejuni*	2m/F	Pneumonia, gastroenteritis, transient bacteremia	Blood	0.07	2	4	ILV^-
84-65	*C. coli*	7m/M	Pneumonia, no diarrhea, transient bacteremia	Blood	0.18	7, 5, 6, 31	52	0
84-29	*C. jejuni*	60/M	Acute cholecystitis	Gallbladder	2.05	30	55	0
84-30	*C. coli*	54/F	Acute cholecystitis	Gallbladder	1.99	52, 33w	30	0
84-59	*C. jejuni*	66/F	Acute cholecystitis	Gallbladder	1.04	28, 29, 1w, 18w	53	0
84-100	*C. jejuni*	77/M	Urinary tract infection, no diarrhea	Urine	0.31	1	2	0
84-24	*C. coli*	Unknown	Unknown	Retroperitoneal abscess	2.32	30, 1w	55	0
84-76	*C. jejuni*	51/M	Chronic renal failure	Peritoneal dialysis fluid	1.29	4, 13w	10, 13	Met^-, Pro^-
84-77	*C. jejuni*	65/F	Ovarian cyst, no diarrhea	Peritoneal (cyst) fluid	0.57	4	1	0
84-99	*C. jejuni*	72/F	Thoracic wall abscess; post-local radiation therapy	Thoracic wall	0.24	1, 44	2	0
84-19	*C. jejuni*	12d/M	Meningitis, hypogammmaglobulinemia	CSF	0.01	13, 16w, 43w	1	Met^-, ILV^-, CC^-
84-25	*C. jejuni*	Child	Meningitis	CSF	0.50	2	4	Met^-, Ser^-, CC^-
84-67	*C. coli*	8m/M	Meningitis, no diarrhea	CSF	0.14	5, 31	32	ND

* Age in years unless otherwise marked. d, days; m, months.

† log_{10} killing in standardized assay [12].

‡ NT, nontypable; w, weak.

§ Pair of isolates from the same patient.

‖ Met^-, requires methionine for growth; Pro^-, requires proline; Arg^-, requires arginine; ILV^-, requires isoleucine, leucine, and valine; CC^-, requires cysteine and cystine; Ser^-, requires serine; ND, not done.

sidered to be clinically significant. Of 13 bloodstream isolates, seven were considered to be transient, in that the patient usually had a brief febrile period at the height of the diarrheal illness, and the bacteremia cleared with no or inappropriate antibiotics or oral erythromycin. Of the other six isolates, five caused illnesses consistent with gram-negative sepsis, and one patient had a septic abortion.

Serum susceptibility. Of 13 isolates from the bloodstream included in this study, six (46%) were serum sensitive, whereas the remaining seven were intermediately or completely resistant (figure 1). We studied isolates from both the feces and blood from two patients. In each case, the susceptibilities of the blood and fecal isolates were similar, but one pair was serum sensitive and the other was serum resistant (table 1). Of 11 other isolates from extraintestinal sources, five were serum sensitive, five were intermediate, and one was resistant. Four of these extraintestinal isolates (three from gallbladder, one from urine) probably reached their site by direct extension from the gastrointestinal tract. Three (75%) of these were serum sensitive. Five other isolates (three from CSF, one from thoracic wall abscess, one from peritoneal fluid) probably were hematogenous; none was serum sensitive. For two other isolates, from an ovarian cyst and from a retroperitoneal abscess, either direct extension from the gastrointestinal tract or hematogenous spread was possible; both isolates were serum sensitive. Two of the isolates from CSF that were serum resistant in the standard assay were then incubated for 240 min with 67% serum [12]. Under these more stringent conditions both remained highly serum resistant; one isolate (84-19) showed no kill at all while numbers of the other (85-3) were only minimally reduced (0.29 $\log_{10}$ killing). For comparison, we have studied 14 fecal strains of *C. jejuni* from patients with gastroenteritis from whom extraintestinal isolations were not made. The distribution of serum susceptibilities of the fecal isolates was nearly identical to that of the blood isolates (figure 1). When isolates were grouped by origin, the systemic isolates (blood plus hematogenous) were relatively more serum resistant than the gastrointestinal tract isolates (fecal plus direct extension) although there was considerable overlap. Isolates from seven patients with transient bacteremias were among the least serum susceptibile (median, 0.22 $\log_{10}$ kill).

From two patients, more than one bloodstream isolation of *C. jejuni* was made. The first patient was a normal host who had bacteremia at the same time

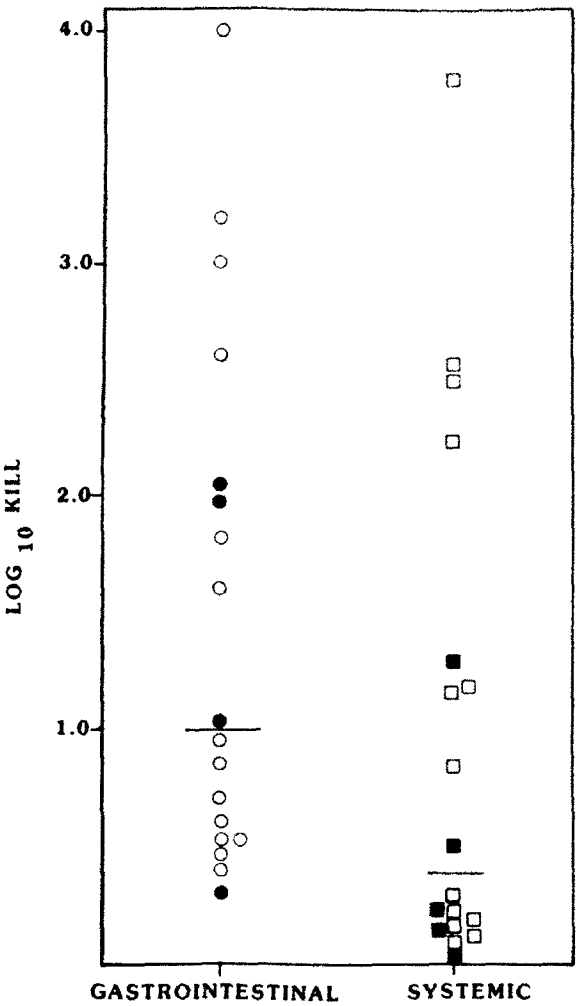

Figure 1. Susceptibility of *C. jejuni* and *C. coli* strains to normal human serum by isolation site. Gastrointestinal strains include 14 fecal isolates (○) and 4 strains isolated from sites directly contiguous to the gastrointestinal tract (●; see text). Systemic strains include 13 blood isolates (□) and 5 strains isolated from sites not directly contiguous to the gastrointestinal tract (■; see text). Serum susceptibility was defined as 1.0 $\log_{10}$ killing in a standardized assay [12]. The solid line for each column represents the median.

he had colitis and was found to be bacteremic 13 days later, when he was asymptomatic [3]. His isolate (84-28) was serum resistant (0.19 $\log_{10}$ kill). The second patient had systemic lupus erythematosus and had recurrent *Campylobacter* diarrhea and bacteremia [5]. She was hypogammaglobulinemic with deficient serum bactericidal activity against either her isolate or a heterologous isolate known to be exquisitely serum sensitive [5]. An isolate of her infective organism was serum sensitive (1.16 $\log_{10}$ kill). Strains iso-

lated from the 12 patients with predispositions for extraintestinal spread were more often serum sensitive (75%) than strains isolated from 11 patients without such predispositions (9%; $P = .002$ by Fisher's exact test). In a similar analysis, isolates from patients with no predisposition to extraintestinal infection were more likely to be serum resistant (mean $\log_{10}$ kill $= 0.64 \pm 0.32$) than were isolates from patients with predispositions (1.38 ± 0.26; $P = .05$ by one-tailed t test).

SDS-PAGE. There were no consistent differences in protein bands resolved in whole-cell preparations of four serum-sensitive and eight serum-resistant strains of *C. jejuni*. Twelve to 16 bands between 43,000 and 200,000 were resolved for each strain, but the serum-sensitive strains possessed each of the bands seen for the serum-resistant strains (data not shown). Using proteinase-K-treated whole-cell lysates, we studied LPS structure of the isolates from blood and the systemic isolates. Recently we showed that serum-sensitive *C. jejuni* fecal isolates all had rough-type LPS [15, 16]. All of 23 extraintestinal strains studied, which had various serum susceptibilities, also showed rough-type LPS (figure 2). Extending developing time of the silver stain to 1 hr (not shown) did not result in visualization of high-molecular-weight banding such as is seen for *Campylobacter fetus* LPS [15].

Chemical analysis. We determined total cell protein, carbohydrate, and KDO concentrations for 10 fecal isolates, 10 isolates from blood, and 11 other extraintestinal isolates (table 2). For the purposes of this analysis, we also used the systemic and gastrointestinal categories defined above, which collectively include the 11 extraintestinal isolates. Total concentrations of carbohydrate were significantly lower and the KDO:carbohydrate ratios higher in gastrointestinal isolates than in systemic isolates. For the total of 31 strains from all sites, we examined the relation between $\log_{10}$ killing and these chemical characteristics. By linear-regression analysis, serum susceptibility was inversely correlated with total carbohydrate ($r = -.46$, $P = .01$) and KDO ($r = -.51$, $P = .003$) concentrations and directly correlated with KDO:carbohydrate ratio ($r = .52$, $P = .003$). There was no correlation between serum susceptibility and protein concentration.

Serotyping and auxotyping. A wide variety of serotypes were seen among the 24 isolates from the bloodstream and other extraintestinal sites on the basis of both heat-stable and heat-labile antigens (table 1). Nevertheless, PEN serotype antigens 1 through 4 were present in 13 (54%) of 24 isolates, and Lior serotype antigens 1 through 4 were present in 11 (46%) of 24 isolates. Isolates from patients with or without predispositions to systemic infection were equally likely to be of common serotypes. An isolate of *C. jejuni* (84-133) from a culture of blood ob-

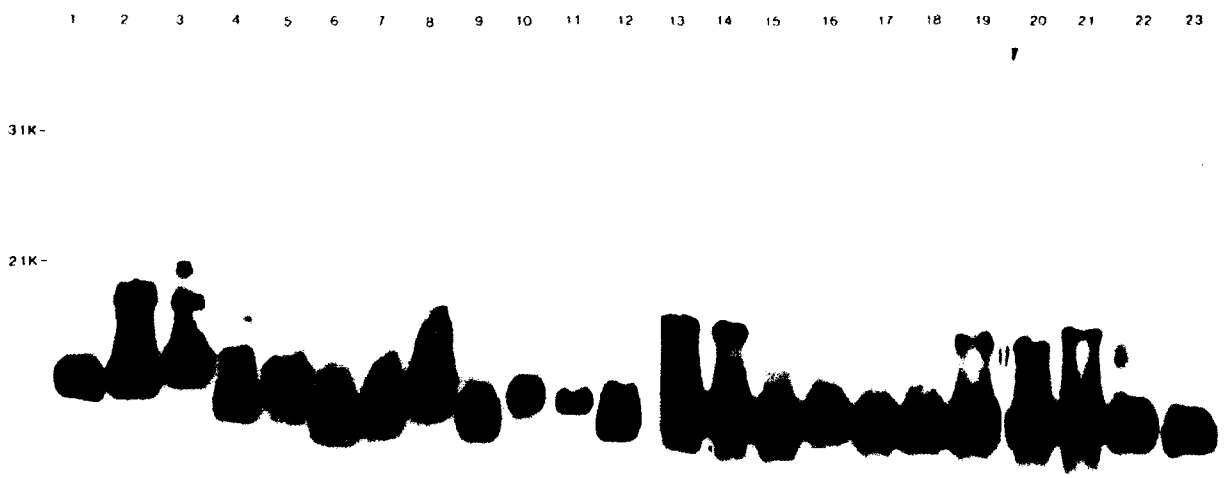

Figure 2. PAGE in 15% acrylamide of proteinase-K-treated whole-cell lysates of *C. jejuni* and *C. coli* isolates from the bloodstream and other extraintestinal sites. Lysates were prepared, loaded onto PAGE, and silver stained as previously described [15, 16]. Strains examined in lanes are: 1, 84-59, 2, 84-65; 3, 84-66; 4, 84-67; 5, 84-68; 6, 84-76; 7, 84-77; 8, 84-99; 9, 84-100; 10, 84-101; 11, 84-102; 12, Holland; 13, 84-23; 14, 84-24; 15, 84-25; 16, 84-26; 17, 84-27; 18, 84-28; 19, 84-29; 20, 84-30; 21, 84-133; 22, 84-134; 23, 84-157.

Table 2. Chemical composition of 31 strains of *C. jejuni* or *C. coli* by isolation site.

Site	No. studied	Percent of dry cell weight as Protein	CHO	KDO	KDO:CHO	Log_{10} kill
Fecal	10	18.9 ± 1.7	0.6 ± 0.14	0.16 ± 0.02	0.32 ± 0.07	1.58 ± 0.42
Gastrointestinal*	14	19.5 ± 1.5	0.6 ± 0.11	0.16 ± 0.01	0.32 ± 0.06	1.51 ± 0.31
Blood	10	23.4 ± 2.0	1.4 ± 0.20	0.18 ± 0.01	0.14 ± 0.03	0.80 ± 0.29
Systemic†	15	23.1 ± 1.5	1.4 ± 0.12‡	0.18 ± 0.01	0.14 ± 0.02§	0.63 ± 0.20‖
All sites**	31	21.8 ± 1.1	1.1 ± 0.11	0.17 ± 0.01	0.22 ± 0.03	1.10 ± 0.19

NOTE. Data are mean ± SE. CHO, carbohydrate; KDO, ketodeoxyoctonate.

* Includes 10 fecal isolates and 4 extraintestinal isolates due to direct extension (see text).

† Includes 10 blood isolates and 5 extraintestinal isolates of hematogenous origin (see text).

‡ Compared with gastointestinal isolates, $P = .00004$.

§ Compared with gastrointestinal isolates, $P = .004$.

‖ Compared with gastrointestinal isolates, $P = .02$.

** Includes 2 extraintestinal isolates that were not classified.

tained 72 hr after a fecal isolate (84-66) from the same person had essentially identical serotypes in both systems. However, a blood isolate (79-263) obtained three weeks after a fecal isolate (79-193) from a patient with acute colitis was of a different serotype. In both cases, bacteremias were transient. Sixteen of the 24 isolates demonstrated no auxotrophic requirements. There was no apparent correlation between the site of isolation and the auxotype of the strain.

Discussion

One of the most consistent features of *C. jejuni* and *C. coli* is the ability to cause illness in normal but nonimmune hosts [1]. In numerous instances of endemic and epidemic *C. jejuni* infection, the vast preponderance of affected individuals were previously healthy [1, 2, 19–21]. *C. jejuni* bacteremia appears to be uncommon; however, the infrequency with which blood cultures are obtained in patients with diarrheal illnesses, and the relative difficulty in isolating *C. jejuni* from blood culture systems (W.-L. L. Wang and M.J. Blaser, unpublished data) may partially explain this phenomenon. In contrast, a closely related organism, *Campylobacter fetus* ssp. *fetus* most frequently is isolated from compromised hosts, most often from the bloodstream and other systemic sites [22]. Although most *C. fetus* isolations from the bloodstream and other systemic sites are clinically significant, transient bacteremias are noted as well. Essentially all isolates of *C. fetus* are serum resistant [12, 23].

Recent CDC surveillance data indicated that 11 of 21 isolates of *C. jejuni* from blood but only 15% of 5,471 isolates from stool were from patients at the extremes of age [7]; we also found that >50% of patients with extraintestinal infections were at the extremes of age. Similarly, in our series, 52% of the patients with extraintestinal infections had a predisposition or diathesis for such infection. These included disorders of immunologic function, such as hypogammaglobulinemia, early infancy, and chronic renal failure, and also localized host defects such as biliary tract disease, radiation therapy, and aortic prostheses. Therefore in such patients, extraintestinal spread of *C. jejuni* or *C. coli* should be considered opportunistic. In support of that hypothesis was our finding that most strains from hosts with predispositions to systemic infections were serum sensitive, whereas only one strain from a normal host was serum sensitive. These findings suggest that for extraintestinal infection to occur in a normal host, increased virulence must be present. *Campylobacter*-like organisms have been associated with diarrheal illness in homosexual men [24], and type strains studied in our laboratory were serum sensitive [12]. That the first reported extraintestinal isolates [25] of these presumably serum-sensitive strains were from immunocompromised hosts is consistent with the findings in our study. That all strains isolated from deep infections that were due to hematogenous spread were serum resistant further supports the hypothesis that serum resistance is an important virulence factor permitting systemic dissemination of pathogens present on mucosal surfaces in normal hosts [26, 27].

Extraintestinal isolates of *C. jejuni* and *C. coli*

resembled fecal isolates of these same organisms in several ways. Common auxotypes and heat-stable and heat-labile serotypes among fecal isolates [13, 14, 14a, 28] also were common among extraintestinal isolates [9]. All strains had rough-type LPS profiles on PAGE, and the distribution of serum susceptibility among fecal, bloodstream, and other extraintestinal isolates as a whole were nearly the same. Nevertheless, the subgroup of systemic isolates was significantly different from gastrointestinal isolates in that they were less serum sensitive and differed in carbohydrate content and KDO:carbohydrate ratio (table 2).

The mechanisms for resistance of *C. jejuni* to the complement- and specific antibody–mediated activity in normal human serum [12] are not known. For other gram-negative organisms with rough LPS, the presence of specific outer membrane proteins is associated with serum resistance [29]. Using SDS-PAGE, we found no evidence for this phenomenon for *C. jejuni*, and our chemical analysis did not show any relation between whole-cell protein content and susceptibility to killing. For *C. fetus*, serum resistance is partially associated with smooth-type LPS [15, 16], but such is not the case for *C. jejuni*. All strains studied show a rough-type LPS structure with low concentrations of high-molecular-weight polysaccharide side-chain complexes. Our chemical analyses indicate that serum resistance is associated with increased carbohydrate fraction of total cell weight. This result suggests the presence of a capsule on resistant strains or a preference to produce more LPS molecules or longer polysaccharide side chains. That KDO fraction was inversely related to serum susceptibility suggests that more resistant strains produce more LPS molecules. However, that the ratio of KDO to total carbohydrate was directly related to serum susceptibility suggests that resistant strains could have a tendancy to produce longer polysaccharide side chains when compared with sensitive strains. For the Enterobacteriaceae, relatively rough strains are more serum sensitive [30] and less virulent [26] than (smooth) strains with long polysaccharide side chains, and among smooth strains, polysaccharide composition affects virulence [15, 31]. Differences in specific chemical composition and biological activity of lipopolysaccharides [32] also could affect ability to invade the bloodstream, but these characteristics were not examined in this study. On the basis of our data, we can not presently rule out the presence of a carbohydrate capsule contributing to serum resistance in some strains.

In summary, isolation of extraintestinal *C. jejuni* or *C. coli* appears to be due to at least three distinct phenomena: (*1*) enteric infection in a normal host causing transient bacteremia and clinically mild illness, (*2*) a serum-resistant isolate in a normal host causing sustained bacteremia or focal infection, or (*3*) a host with either a total or a local defect that permits either serum-sensitive or serum-resistant strains to disseminate. The degree to which each of these problems contribute to extraintestinal infection will require further study.

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